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AstraZeneca AB
SE-151 85 Sodertalje
Sweden

Patents ADP number *(if you know it)* 7822448003

If the applicant is a corporate body, give the country/state of its incorporation — Sweden

4. Title of the invention

5. Name of your agent *(if you have one)* — Allen Frank GILES

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AstraZeneca UK Limited
Global Intellectual Property
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METHOD

This invention relates to use of polymorphisms in human OATP-C in statin therapy because they are associated with an effect on statin pharmacokinetics (PK) in humans, especially rosuvastatin pharmacokinetics. The invention also relates to the use of OATP-C polymorphisms in predicting the efficacy and safety of statins, whose uptake in to the liver is mediated by OATP-C, especially rosuvastatin.

The OATP-C gene (sometimes called OAPT2) has been cloned by four different groups, annotated and published as EMBL accession numbers AB026257 (OATP-C, 2452bp), AF205071(OATP2, 2830), AJ132573(OATP2, 2778), and AF060500 (LST-1). Konig (2000) J Biol Chem 275, 23161-23168 describes the genomic organisation of OATP 1, 2 and 8. International patent application WO 00/08157 describes human anion transporter genes and some polymorphisms.

Na+-independent organic anion transporting polypeptide (OATP) C gene is a member of the OATP supergene family involved in multifunctional transport of organic anions. OATP-C transports a diverse range of molecules e.g. the organic anion taurocholate, conjugated steroids: DHEAS, estradiol 17β-D-glucoronide and estrone-3-sulfate, eicosanoids: PGE_2, thromboxane B_2, leukotriene C_4, and E_4, and thyroid hormones T4 and T3. OATP-C has also been shown to be involved in the transport of xenobiotics , and drugs involved in lipid lowering *e.g.* statins. Statins are a class of drugs which inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA). They are an important therapy for patients with atherosclerotic vascular diseases but some rare adverse events have been noted and one statin (cerivastatin) withdrawn from the market. Pharmacokinetic differences between statins have been associated with prevalence of adverse events (Igel (2002) J Clin Pharmacol **42**: 835-45).

Pravastatin is actively transported from the circulation to the liver via the OATP-C transporter (Hsiang, B. Journal of Biological Chemistry. 274(52), 37161-37168. 1999). Rosuvastatin was shown to be a substrate for OATP-C *in vitro* (Brown (2001) Atherosclerosis Supplements **2**, pg 90, poster abstract P174). Numerous polymorphisms in OATP-C have been reported in the literature and SNP databases. Identification of SNPs in OATP-C was reported in EP1186672. Polymorphism in OATP-C has been reported by Tamai *et al* (2000), BBRC, **273**, 251-60 and reviewed by Tirona (2002) Adv Drug Deliery Reviews 54:1343-52. Tirona showed that some OATP-C polymorphisms, including the V174A variant, were associated with reduced transport of endogenous substrates *in vitro*. Using a different cell

system, Nozawa et al (2001) J Pharmacol Exp Ther, **302**, 804-13, found that the V174A (OATPC*5) variant did not affect substrate transport. Tirona stated that the *in vivo* relevance of OATP-C polymorphisms remained to be determined. Nishizato (2003) Clin Pharmacol Ther 73:554-65 published *in vivo* data in Japanese healthy volunteer humans showing that the OATPC*15 allele, containing both the N130D and the V174A polymorphisms, had an effect on the pharmacokinetics of pravastatin in healthy volunteers. Nishizato did not report the effect of the OATP-C*5 allele, which has not been detected in the Japanese population to date, and stated that large clinical studies are needed. There have been no pharmacokinetic studies in patients taking pravastatin. There have been no pharmacogenetic studies on healthy volunteers or patients taking rosuvastatin. Hence, the observations of Nishizato et al performed in Japanese healthy volunteers are not predictive of the PK profiles of patients or of other populations. Population PK modelling analyses, using data collected by AstraZeneca in the rosuvastatin clinical development programme, confirm that healthy volunteers and patients differ with respect to their distribution of rosuvastatin. Patients receiving statins for lipid lowering may be on other drugs transported by OATP-C and drug-drug interactions may affect the PK profile of statins (Int J Clin Pharmacol Ther (2002), 40, 439-50). Patients prescribed statins may also have other liver and kidney complications affecting the distribution and excretion of statins. There is an entire chapter in the textbook Clinical Pharmacokinetics (Chapter 16, pages 248-266 in 3rd edition 1995, Rowland & Tozer, published by Williams & Wilkins) which begins:

"Disease is a major source of variability in drug response. For many diseases this is due primarily to differences in pharmacokinetics..."

Hence there is a need to identify which polymorphisms in OATP-C have an effect on *in vivo* pharmacokinetics of rosuvastatin and other statins in patients with vascular disease or a predisposition thereto. Our invention is based on the discovery that the V174A polymorphism has a statistically significant effect on rosuvastatin pharmacokinetics in patients. The V174A polymorphism may affect the efficacy and safety of statins, especially rosuvastatin.

According to one aspect of the present invention there is provided an in vitro diagnostic method to identify a patient at risk of developing a side effect whilst on statin therapy or to identify a patient potentially requiring a statin dose level above the minimum recommended dose level in which the method comprises testing a biological sample from the

patient for presence of alanine at position 174 of OATP-C polypeptide and/or a polymorphism in linkage disequilibrium therewith.

The biological sample is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before analysis of allelic variation. Preferably the patient is tested for presence of alanine at position 174 either through analysis of polypeptide directly or through analysis of genetic material encoding the polypeptide. As patients carry 2 copies of the OATPC gene they may be homozygous or heterozygous genotype. Polymorphisms in linkage disequilibrium with alanine at 174 may be tested as an alternative to determining the presence of alanine at 174 directly.

It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system. Table 1 lists a number of mutation detection techniques, some based on the PCR. These may be used in combination with a number of signal generation systems, a selection of which is listed in Table 2. Further amplification techniques are listed in Table 3. Many current methods for the detection of allelic variation are reviewed by Nollau *et al.*, Clin. Chem. **43**, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

Abbreviations:

ALEX™	Amplification refractory mutation system linear extension
APEX	Arrayed primer extension
ARMS™	Amplification refractory mutation system
b-DNA	Branched DNA
bp	base pair
CMC	Chemical mismatch cleavage
COPS	Competitive oligonucleotide priming system
DGGE	Denaturing gradient gel electrophoresis

FRET	Fluorescence resonance energy transfer
LCR	Ligase chain reaction
MASDA	Multiple allele specific diagnostic assay
NASBA	Nucleic acid sequence based amplification
OLA	Oligonucleotide ligation assay
PCR	Polymerase chain reaction
PTT	Protein truncation test
RFLP	Restriction fragment length polymorphism
SDA	Strand displacement amplification
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism analysis
SSR	Self sustained replication
TGGE	Temperature gradient gel electrophoresis

Table 1 - Mutation Detection Techniques

General: DNA sequencing, Sequencing by hybridisation

Scanning: PTT*, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage

* Note: not useful for detection of promoter polymorphisms.

Hybridisation Based

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips)

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, **14**, 303; WO 95/13399 (Public Health Inst., New York)

Extension Based: ARMS™, ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, **17**, 2347.

Incorporation Based: Mini-sequencing, APEX

Restriction Enzyme Based: RFLP, Restriction site generating PCR

Ligation Based: OLA

Other: Invader assay

Table 2 - Signal Generation or Detection Systems

Fluorescence: FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)

Other: Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric, Hybridisation protection assay, Mass spectrometry

Table 3 - Further Amplification Methods

SSR, NASBA, LCR, SDA, b-DNA

Table 4- Protein variation detection methods

Immunoassay

Immunohistology

Peptide sequencing

Preferred mutation detection techniques include ARMS™, ALEX™, COPS, Taqman, Molecular Beacons, RFLP, and restriction site based PCR and FRET techniques. Immunoassay techniques are known in the art e.g. A Practical Guide to ELISA by D M Kemeny, Pergamon Press 1991; Principles and Practice of Immunoassay, 2nd edition, C P Price & D J Newman, 1997, published by Stockton Press in USA & Canada and by Macmillan Reference in the United Kingdom. Histological techniques are described in Theory and Practice of Histological Techniques by J D Bancroft & A Stevens, 4th Edition, Churchill Livingstone,1996. Protein sequencing is described in Laboratory Techniques in Biochemistry and Molecular Biology, Volume 9, Sequencing of Proteins and Peptides, G Allen, 2nd revised edition, Elsevier, 1989.

Particularly preferred methods include ARMS™ and RFLP based methods. ARMS™ is an especially preferred method.

Antibodies can be prepared using any suitable method. For example, purified polypeptide may be utilized to prepare specific antibodies. The term "antibodies" is meant to include polyclonal antibodies, monoclonal antibodies, and the various types of antibody constructs such as for example $F(ab')_2$, Fab and single chain Fv. Antibodies are defined to be specifically binding if they bind the allelic variant of CPB2 with a K_a of greater than or equal to about 10^7 M^{-1}. Affinity of binding can be determined using conventional techniques, for example those described by Scatchard et al., *Ann. N.Y. Acad. Sci.*, 51:660 (1949).

Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice or rats, using procedures that are well-known in the art. In general, antigen is administered to the host animal typically through parenteral injection. The immunogenicity of antigen may be enhanced through the use of an adjuvant, for example, Freund's complete or incomplete adjuvant. Following booster immunizations, small samples of serum are collected and tested for reactivity to antigen. Examples of various assays useful for such determination include those described in: *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; as well as procedures such as countercurrent immuno-electrophoresis (CIEP), radioimmunoassay, radioimmunoprecipitation, enzyme-linked immuno-sorbent assays (ELISA), dot blot assays, and sandwich assays, see U.S. Patent Nos. 4,376,110 and 4,486,530.

Monoclonal antibodies may be readily prepared using well-known procedures, see for example, the procedures described in U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439 and 4,411,993; Monoclonal Antibodies, Hybridomas: *A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKearn, and Bechtol (eds.), (1980).

Monoclonal antibodies can be produced using alternative techniques, such as those described by Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas", *Strategies in Molecular Biology* 3: 1-9 (1990) which is incorporated herein by reference. Similarly, binding partners can be constructed using recombinant DNA techniques to incorporate the variable regions of a gene that encodes a specific binding antibody. Such a technique is described in Larrick et al., *Biotechnology*, 7: 394 (1989).

Once isolated and purified, the antibodies may be used to detect the presence of particular polypeptide variants in a sample using established assay protocols, see for example "A Practical Guide to ELISA" by D. M. Kemeny, Pergamon Press, Oxford, England.

Statins already approved for use in humans include atorvastatin, cerivastatin, fluvastatin, pravastatin and simvastatin. The reader is referred to the following references for further information: Drugs and Therapy Perspectives (12th May 1997), **9**: 1-6; Chong (1997) Pharmacotherapy **17**: 1157-1177; Kellick (1997) Formulary **32**: 352; Kathawala (1991) Medicinal Research Reviews, **11**: 121-146; Jahng (1995) Drugs of the Future **20**: 387-404, and Current Opinion in Lipidology, (1997), **8**, 362 - 368. A preferred statin drug is compound 3a (S-4522) in Watanabe (1997) Bioorganic and Medicinal Chemistry **5**: 437-444;

now called rosuvastatin, see Olsson (2001) American Journal of Cardiology, 87, supplement 1, 33-36.

Preferably the statin is rosuvastatin. Preferably the patient is prescribed at least 40mg of rosuvastatin daily, more preferably the patient is prescribed at least 60mg of rosuvastatin daily and especially the patient is prescribed at least 80mg of rosuvastatin daily.

Preferably the patient is additionally tested for presence of valine at position 174 of OATP-C polypeptide whereby presence of both valine and alanine at position 174 indicates heterozygosity at this locus.

Preferably the polymorphism in linkage disequilibrium with alanine174 OATP-C is selected from at least one of:

a) Asp130 OATP-C; or

b) consensus NF1 transcription factor binding sites at positions –26A>G or –118A>C relative to the transcription initiation site; or

c) -309T>C, -878A>G, -903C>T, -1054G>T, -1215T>A or –1558 T>C, where nucleotide positions are relative to the transcription initiation site; or

d) polymorphisms in the 3'UTR region of the OATP-C gene selected from T2122G, C2158T, A2525C, and G2651A, where the nucleotide position is relative to the ATG.

In one embodiment the biological sample is tested for presence of an amino acid at a position of OATP-C polypeptide through analysis of genetic material encoding the polypeptide.

Another aspect of the invention provides an in vitro method of monitoring a patient for a side effect related to statin therapy wherein the method comprises testing a biological sample from the patient for a parameter indicative of a side effect and wherein the patient is selected for such monitoring by a method described herein.

Preferably the side effect is selected from myopathy and renal function. Chemistry tests of renal function include Serum Creatinine (Cr), Blood Urea Nitrogen (BUN), BUN to Serum Creatinine Ratio, Creatinine Clearance and Fractional Excretion of Sodium (FENa). Signs of myopathy include muscle ache, pain, shortness of breath which may be followed by a muscle tissue biopsy to determine abnormalities suggestive of myopathy.

Preferably the patient is OATPC*5 or *15 genotype.

According to another aspect of the present invention there is provided a method for the detection of a polymorphism in OATPC in a human, which method comprises determining the sequence of the human at at least one of the following polymorphic positions:

a) consensus NF1 transcription factor binding sites at positions –26A>G or –118A>C relative to the transcription initiation site; or

b) -309T>C, -878A>G, -903C>T, -1054G>T, -1215T>A or –1558 T>C, where nucleotide positions are relative to the transcription initiation site; or

c) polymorphisms in the 3'UTR region of the OATP-C gene selected from T2122G, C2158T, A2525C, and G2651A, where the nucleotide position is relative to the ATG.

According to another aspect of the present invention there is provided a human OATPC gene or its complementary strand comprising a variant allelic polymorphism at one or more of positions defined herein or a fragment thereof of at least 20 bases comprising at least one novel polymorphism.

According to another aspect of the present invention there is provided an allele specific primer capable of detecting a OATPC gene polymorphism, preferably at one or more of the positions as defined herein.

An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for ARMS™ assays. The allele specific primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

An allele specific primer preferably corresponds exactly with the allele to be detected but derivatives thereof are also contemplated wherein about 6-8 of the nucleotides at the 3' terminus correspond with the allele to be detected and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the primer.

Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If required the primer(s) may be labelled to facilitate detection.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a OATPC gene polymorphism, preferably at one or more of the positions defined herein.

The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection.

According to another aspect of the present invention there is provided an allele specific primer or an allele specific oligonucleotide probe capable of detecting a OATPC gene polymorphism at one of the positions defined herein.

According to another aspect of the present invention there is provided a diagnostic kit comprising an allele specific oligonucleotide probe of the invention and/or an allele-specific primer of the invention.

The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase.

According to another aspect of the present invention there is provided a method of treating a patient in need of treatment with a statin in which the method comprises:

i) use of an in vitro diagnostic method to identify a patient at risk of developing a side effect whilst on statin therapy or to identify a patient potentially requiring a dose level above the minimum recommended dose level in which the method comprises testing a biological sample from the patient for presence of alanine at position 174 of OATP-C polypeptide and/or a polymorphism in linkage disequilibrium therewith; and

ii) administering an effective amount of the drug.

According to another aspect of the invention there is provided use of a statin in preparation of a medicament for treating a patient with vascular disease or a predisposition thereto wherein the patient is identified by an in vitro diagnostic method to identify a patient at risk of developing a side effect whilst on statin therapy or to identify a patient potentially requiring a dose level above the minimum recommended dose level in which the method comprises testing a biological sample from the patient for presence of alanine at position 174 of OATP-C polypeptide and/or a polymorphism in linkage disequilibrium therewith.

According to another aspect of the invention there is provided a method of classifying a patient in need of statin therapy comprising testing a biological sample from the patient for presence of alanine at position 174 of OATP-C polypeptide and/or a polymorphism in linkage disequilibrium therewith.

According to another aspect of the invention there is provided a method of identifying a patient on statin therapy that requires side effect monitoring comprising testing a biological sample from the patient for presence of alanine at position 174 of OATP-C polypeptide and/or a polymorphism in linkage disequilibrium therewith.

"Side effect" means a consequence other than the one for which an agent or measure is used, as the adverse effects produced by a drug, especially on a tissue or organ system other than the one sought to be benefited by its administration.

"Adverse event" means an abnormal or harmful effect to an organism caused by exposure to a chemical. It is indicated by some result such as death, a change in food or water consumption, altered body and organ weights, altered enzyme levels, or visible illness. An effect may be classed as adverse if it causes functional or anatomical damage, causes change in the homeostasis of the organism, or increases the susceptibility of the organism to other chemical or biological stress.

"Linkage disequilibrium" means the occurrence of genetic loci together, more often than would be expected.

"Patient" means a person who is receiving medical treatment.

"Dose" means quantity to be administered at one time, such as a specified amount of medication. For rosuvastatin, the adult starting dose is usually 10mg daily. Higher doses may be required to produce desired lipid profiles in some patients.

In rosuvastatin clinical trials population differences in PK profile (possibly a statin class-effect) have been observed between Japanese and Caucasian subjects, and other ethnic groups.

The invention will now be illustrated by the following non-limiting Examples in which:

Figure 1 shows the effect of the V174A polymorphism on plasma levels of rosuvastatin. Correlation between genotype, for 3 non-synonymous SNPs in OATP-C, and dose-normalised plasma rosuvastatin values (ng/ml/mg) illustrates that the Val174Ala variant is associated with higher plasma concentrations. WT = wild-type homozygote, HET = heterozygote, VAR = homozygous variant. None of the 52 subjects analysed were homozygous variant for either

the Val174Ala or the Pro155Thr variants. Of the 52 subjects analysed, 42 were recorded as being of Caucasian origin. The other 10 subjects were either Hispanic, Black or Asian. **Figure 2** shows the effect of the OATP-C*15 haplotype on plasma levels of rosuvastatin. Correlation between OATP-C haplotypes and dose-normalised plasma rosuvastatin values (ng/ml/mg) illustrates that the OATP-C*15 haplotype is associated with higher plasma concentrations. See Table 1 below for a description of amino acid variants for each haplotype. Results for subjects haplotype pairs with n=3 or fewer (*15/*14, *1b/*14, *1b/*15) are not shown.

In Figures 1 and 2, the lower and upper lines of the "box" are the 25th and 75th percentiles of the sample. The distance between the top and bottom of the box is the interquartile range. The line in the middle of the box is the sample median. The "whiskers", extending above and below the box, show the extent of the rest of the sample (unless there are outliers). Assuming no outliers, the maximum of the sample is the top of the upper whisker. The minimum of the sample is the bottom of the lower whisker. By default, an outlier is a value that is more than 1.5 times the interquartile range away from the top or bottom of the box. Individual data points are outliers.

Figure 3 shows the effect of the Val174Ala variant on plasma levels of rosuvastatin in patients treated for 6 weeks with different doses of rosuvastatin. Plasma rosuvastin levels at 6 weeks have been dose normalised for the analysis. Mean plasma rosuvastatin levels were higher in subjects heterozygous for the Val174Ala variant, as compared to homozygous wild-type subjects (Val/Val). The correlation between the V174A variant and plasma rosuvastatin PK levels was most evident at the higher doses of rosuvastatin.

Example 1

Polymorphisms in OATP-C affect the *in vivo* disposition of statins in patients

In brief, the OATP-C gene was sequenced in 79 human clinical trial subjects. 52 of these patients had received rosuvastatin for at least 6 weeks for dislipidaemic disease and had plasma PK measurements taken after 6 weeks of treatment. Data for these 52 patients were used to show that some polymorphic variants in OATP-C have a functionally significant effect on plasma levels of statins.

Methodology

The promoter, exons and 3' untranslated regions of OATP-C were fully sequenced by DNA terminator sequencing in DNA collected from 79 subjects in clinical trials. Sequencing traces were used to record the genotypes for known (i.e. available in the literature or SNP databases) SNPs in OATP-C. Some novel SNPs were found in the promoter and 3'UTR region of OATP-C.

Mean dose-normalised plasma rosuvastatin concentrations were determined for the genotypes for each polymorphic variant in the OATP-C gene. OATP-C genotype data for 3 SNPs, namely amino acid position 130 Asparagine → Aspartic acid (Asn130Asp), 155 Proline → Threonine (Pro155Thr) and 174 Valine→Alanine (Val174Ala), was utilised to determine the haplotype pair for each subject. Mean dose-normalised plasma rosuvastatin concentrations were determined for the subjects grouped by haplotype pair.

All consenting subjects treated with rosuvastatin (n=271), from the 2 clinical trials including the original 52 patients, were subsequently genotyped, using TaqMan™, for OATP-C variants. Data for SNPs causing the N130D, P155T and V174A variants were utilised to assign OATPC haplotype pairs to each subject, as previously described.

Results

The sequencing data revealed a number of SNPs not previously reported, 8 in the promoter region (Jung, D. 2001 Journal of Biological Chemistry. 276(40), 37206-37214) and 4 in the 3'UTR region of OATP-C. These SNPs represent another aspect of the invention. These 12 novel SNPs were identified via sequencing the OATP-C gene in 79 subjects. Of these novel SNPs, 8 SNPs were located in the OATP-C promoter. 2 of these SNPs were located in consensus NF1 transcription factor binding sites at positions –26A>G and –118A>C relative to the transcription initiation site. Other novel upstream SNPs included, -309T>C, -878A>G, -903C>T, -1054G>T, -1215T>A and –1558 T>C, where nucleotide positions are relative to the transcription initiation site as described by Jung et al. A further 4 novel SNPs were located in the 3'UTR region of the OATP-C gene, namely T2122G, C2158T, A2525C, and G2651A, where the nucleotide position is relative to the ATG.

SNPs in the exons, 5'UTR and 3'UTR regions were found to be in strong linkage disequilibrium when analysed in a pair-wise manner to determine d' values. OATP-C genotype data for 3 SNPs Asn130Asp, Pro155Thr and Val174Ala was utilised to determine the haplotypes. The package SNPHAP (Clayton, David SNPHAPv0.2 2002 http://www-gene.cimr.cam.ac.uk/clayton/software/snphap.txt) was used for this analysis. The haplotypes were also predicted using the PHASE package (Stephens, M. 2001 American Journal of

Human Genetics **68**, 978-989) and were found to give the same predicted haplotypes. The haplotypes were found to be concordant with those reported previously (Tirona 2001). The –118 A>C promoter SNP, at the NF1 binding site, was in strong linkage disequilibrium with the Val174Ala coding variant.

Table 1 Common haplotypes in the OATP-C gene

* nomenclature	Amino acid variant(s) on allele	Haplotype Frequency (n=79)
*1a	Asp130 , Pro155 , Val174	57%
*1b	**Asn130** , Pro155 , Val 174	22%
*5	Asp130 , Pro155 , **Ala174**	2%
*14	**Asn130** , **Thr155** , Val174	7.5%
*15	**Asn130** , Pro155 , **Ala174**	11.5%

Table 2 Frequency of the more common non-synonymous OATP-C SNPs (n=79)

Amino Acid	Major allele	Minor allele	SNP	Frequency
130	Asn130	Asp130	A388G	0.41
155	Pro155	Thr 155	C463A	0.08
174	Val174	Ala 174	T521C	0.13

Table 3: Individuals haplotypes for OATP-C

Haplotype Pair	AZ haplotype ID	No of ind (total = 79)	Frequency
*1a/*1a	A	28	35%
*1b/*1b	B	7	9%
*1a/*1b	C	14	18%
*1a/*15	D	13	16%
*1a/*14	E	6	7%
*1b/*15	F	3	4%
*1b/*14	G	3	4%
*1a/*5	H	2	3%
*15/*14	I	3	4%

Table 4

PK GROUP	Val/Val	Val/Ala	Total
HIGH	17	10	27
LOW	22	3	25
Total	39	13	52

Table 4 shows the distribution of genotypes for the V174A variant between subjects classified in to 'high' and 'low' PK groups based on the distribution of rosuvastin plasma PK levels in 2 phase III trials. The high and low sub-groups represent those subjects with PK values in the 10^{th} and 90^{th} percentiles compared to the distribution of plasma PK values observed for the complete trial cohort. The Val/Ala heterozygote genotype is more common in those subjects with 'high' plasma PK levels, and more frequent than would be expected by chance based on the population allele frequency of the V174A variant [chi squared p=0.037 when n=52 (all subjects) and p=0.019 when n=42 (Caucasian subjects only)] and [exact test p=0.055 when n=52 (all subjects) and p=0.043 when n=42 (Caucasian subjects only)].

Sequence and genotype data from 79 subjects was used to determine the allele and haplotype frequencies. Plasma rosuvastatin concentrations were only available for 52 of these 79 subjects and hence were only small numbers of subjects for some of the haplotype pair groups.

In some populations, e.g. Japanese, the V174A variant occurs as the OATPC*15 allele. In other populations, e.g. Caucasians and Hispanics, the V174A variant is observed on both the OATPC*5 allele and the OATPC*15 allele. Frequencies of these alleles differs between populations.

Conclusions

Evidence of an *in vivo* genotype-phenotype relationship has been determined between OATP-C variants and the pharmacokinetic profile of statins, a common class of drugs used in the treatment of hypercholesterolaemia / dyslipidaemia. The observation of higher plasma concentrations of rosuvastatin in patients with the Ala174 OATP-C variant indicates that transport of rosuvastatin by the Ala174 variant is lower than that of the Val174 OATP-C variant. The Ala174 variant thus causes reduced uptake of statins in to the liver and

consequent increased plasma levels. For non-hepatic adverse events, plasma drug concentration is a risk factor for an adverse event. OATP-C variants N130D and P155T do not appear to affect the pharmacokinetic disposition of rosuvastatin.

The genotype-phenotype correlation between OATP-C variants and *in vivo* plasma levels of statins may be utilised to optimise the statin dose, appropriate for each individual, via a diagnostic assay for the SNP or protein variant. Optimisation of the plasma level will be important in subjects that require high doses of statins for adequate lowering of cholesterol levels to the desired threshold.

Evidence that polymorphisms in OATP-C affect the *in vivo* disposition of statins indicates that OATP-C variants may affect the clinical response to statins and other clinically relevant drugs that are transported by OATP-C. Correlation of polymorphisms in OATP-C with end of treatment dose-normalised plasma rosuvastatin concentrations, determined in subjects treated for 6 weeks with different doses of rosuvastatin, have shown that OATP-C variants have a functional effect on the *in vivo* pharmacokinetic disposition of rosuvastatin. Subjects heterozygous for the Val174Ala variant have increased mean plasma concentrations of rosuvastatin as compared to subjects homozygous for the wild-type Val174 variant at amino acid position 174. Subjects with a single copy of the OATP-C*15 allele (heterozygous for the Val174Ala and Asn130Asp variants) were found to have higher mean plasma concentrations than subjects with the OATP-C*1a, OATP-C*1b, and OATP-C*14 haplotypes. The observation of higher concentrations of rosuvastatin in subjects with the Ala174 OATP-C variant indicates that transport by the Ala174 variant is lower than that of the Val174 OATP-C variant. The Ala174 variant causes reduced uptake of statins in to the liver and has an impact on the clinical response to statins. OATP-C variants affecting the pharmacokinetic profile of statins may be associated with an increased risk of adverse drug reactions to statins as a result of the high concentrations of statins in the circulation. The genotype-phenotype correlation between OATP-C variants and *in vivo* plasma levels of statins may be utilised to optimise the statin dose, appropriate for each individual, via a diagnostic assay for the SNP or protein variant. Optimisation of the plasma level will be important in subjects that require high doses of statins for adequate lowering of cholesterol levels to the desired threshold.

Claims

1 An in vitro diagnostic method to identify a patient at risk of developing a side effect whilst on statin therapy or to identify a patient potentially requiring a dose level above the minimum recommended dose level in which the method comprises testing a biological sample from the patient for presence of alanine at position 174 of OATP-C polypeptide and/or a polymorphism in linkage disequilibrium therewith.

2 A method according to claim 1 wherein the statin is rosuvastatin.

3 A method according to claim 2 wherein the patient is prescribed at least 40mg of rosuvastatin daily.

4 A method according to claim 3 wherein the patient is prescribed at least 60mg of rosuvastatin daily.

5 A method according to claim 3 wherein the patient is prescribed at least 80mg of rosuvastatin daily.

6 A method according to any preceding claim wherein the patient is additionally tested for presence of valine at position 174 of OATP-C polypeptide whereby presence of both valine and alanine at position 174 indicates heterozygosity at this locus.

7 A method according to any preceding claim wherein the polymorphism in linkage disequilibrium with alanine174 OATP-C is selected from at least one of:

a) Asp130 OATP-C; or

b) consensus NF1 transcription factor binding sites at positions –26A>G or –118A>C relative to the transcription initiation site; or

c) -309T>C, -878A>G, -903C>T, -1054G>T, -1215T>A or –1558 T>C, where nucleotide positions are relative to the transcription initiation site; or

d) polymorphisms in the 3'UTR region of the OATP-C gene selected from T2122G, C2158T, A2525C, and G2651A, where the nucleotide position is relative to the ATG.

8 A method according to any preceding claim wherein the biological sample is tested for presence of an amino acid at a position of OATP-C polypeptide through analysis of genetic material encoding the polypeptide.

9 An in vitro method of monitoring a patient for a side effect related to statin therapy wherein the method comprises testing a biological sample from the patient for a parameter indicative of a side effect and the patient is selected for such monitoring by a method according to any preceding claim.

10 A method according to claim 9 wherein the side effect is selected from myopathy and renal function.

11 A method according to any preceding claim wherein the patient is OATPC*5 or *15 genotype.

12 A method of treating a patient in need of treatment with a statin in which the method comprises:

i) use of an in vitro diagnostic method to identify a patient at risk of developing a side effect whilst on statin therapy or to identify a patient potentially requiring a dose level above the minimum recommended dose level in which the method comprises testing a biological sample from the patient for presence of alanine at position 174 of OATP-C polypeptide and/or a polymorphism in linkage disequilibrium therewith; and

ii) administering an effective amount of the statin.

Figure 1

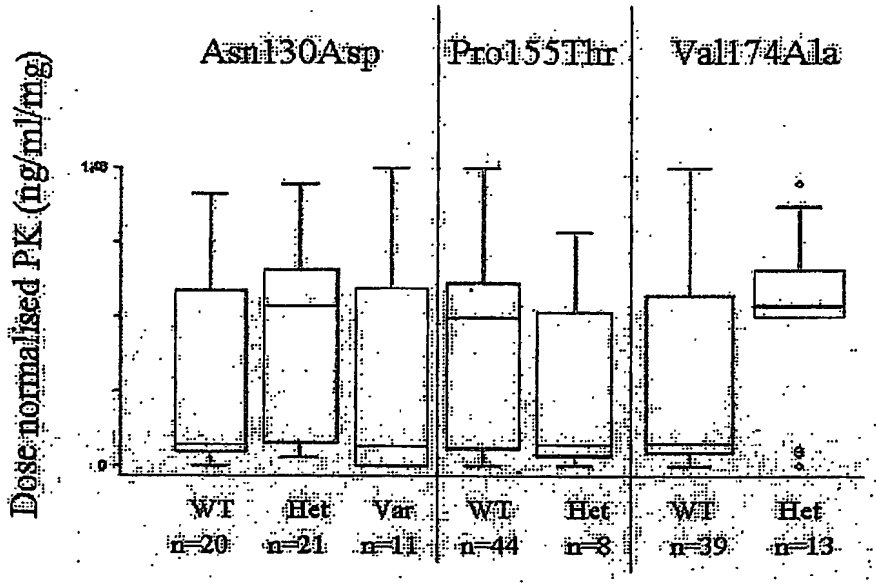


Figure 2

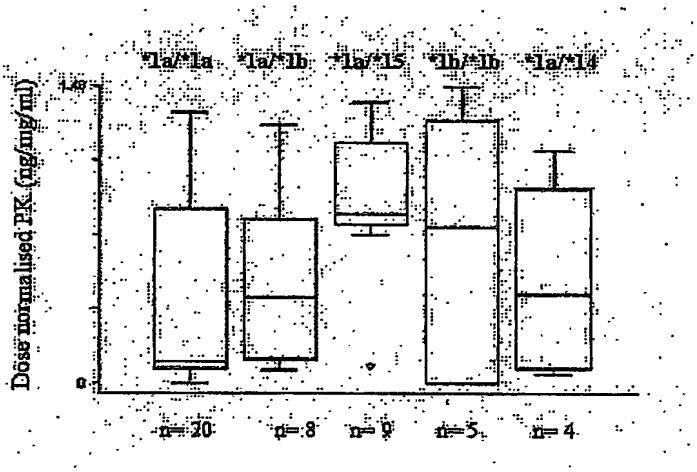
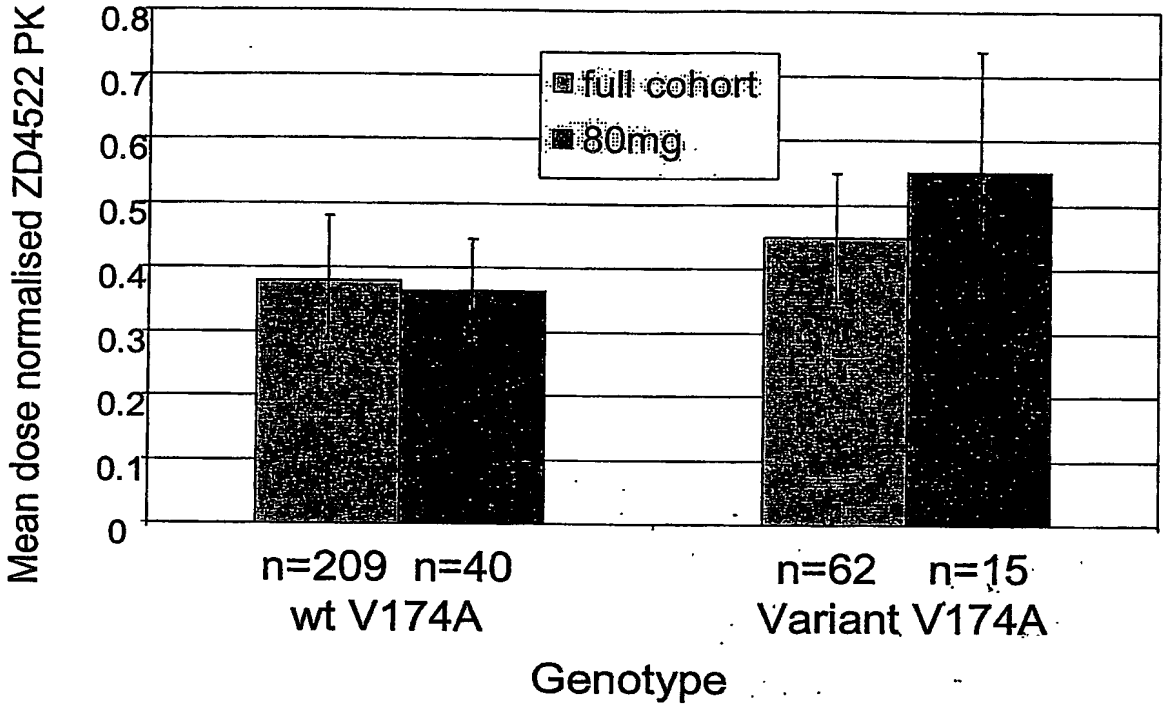


Figure 3


Mean dose normalised ZD4522 PK
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
full cohort
80mg
n=209 n=40
wt V174A
n=62 n=15
Variant V174A
Genotype

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